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# Hexachlorobenzene Uptake by Fathead Minnows and Macroinvertebrates in Recirculating Sediment/Water Systems

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Fathead minnow (*Pimephales promelas*), the worm, *Lumbriculus variegatus*, and the amphipods *Hyalella azteca* and *Gammarus lacustris* were exposed to hexachlorobenzene (HCB) in water with and without a bed of HCB-spiked sediment. Water HCB concentrations were maintained by recirculation through HCB-packed columns. Recirculating HCB-bound particulates and possibly eroded HCB particulates were an added source of HCB in addition to the sediment bed. Significant bioaccumulation of HCB in animal tissues was observed in water-only and water-sediment exposures. The presence of the HCB-spiked sediment did not result in a significant increase in the uptake of HCB by the organisms, but there was a substantial increase in sediment HCB levels over time. Higher tissue HCB levels in aquaria without sediment suggest that the sediment was a more efficient sink for HCB than the organisms.

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# Hexachlorobenzene Uptake by Fathead Minnows and Macroinvertebrates in Recirculating Sediment/Water Systems

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**Abstract.** Fathead minnows (*Pimephales promelas*), the worm, *Lumbriculus variegatus*, and the amphipods *Hyalella azteca* and *Gammarus lacustris* were exposed to hexachlorobenzene (HCB) in water with and without a bed of HCB-spiked sediment. Water HCB concentrations were maintained by recirculation through HCB-packed columns. Recirculating HCB-bound particulates and possibly eroded HCB particulates were an added source of HCB in addition to the sediment bed. Significant bioaccumulation of HCB in animal tissues was observed in water-only and water-sediment exposures. The presence of the HCB-spiked sediment did not result in a significant increase in the uptake of HCB by the organisms, but there was a substantial increase in sediment HCB levels over time. Higher tissue HCB levels in aquaria without sediment suggest that the sediment was a more efficient sink for HCB than the organisms.

Contaminated sediments can be a persistent source of nonpolar chlorinated hydrocarbons for aquatic biota for extended periods (Larsson 1985, 1986). The bioavailability of these sediment-adsorbed organic compounds is linked to solubility, sediment size, sediment organic carbon, type and size of organisms, feeding habits, and extent of direct contact (Neff 1984).

The importance of the predominant exposure route to the biota, whether sediment bed, suspended sediment, pore water, or overlying water is of concern. Adams *et al.* (1985) concluded that the key route of uptake for Kepone® in the midge, *Chironomus tentans*, was the interstitial water and/or the water at the sediment/water interface and not sediment exposure itself. In contrast, Ekelund *et al.* (1987) reported that the ingestion of contaminated sediments by bivalves can also significantly contribute to bioaccumulation.

The purpose of this study was to evaluate the relative importance of freshwater sediments in the uptake and bioconcentration of hexachlorobenzene (HCB) by a variety of organisms in recirculating laboratory systems of equal HCB water concentration in the presence or absence of HCB-spiked sediment. Hexachlorobenzene was selected because of its low toxicity to aquatic organisms (Carlson and Kosian 1987) and low degradability (Scheele 1980). It is used as an agricultural fungicide and also occurs as a waste product in the manufacture of many industrial chemicals (Courtney 1979). It is representative of organic compounds with high octanol-water partition coefficients and low water solubilities. Carlson and Kosian (1987) concluded that HCB at or below saturation levels may not cause toxicological problems in aquatic animals. Tests in our laboratory (A. V. Nebeker, unpublished data) have also demonstrated no toxicity at 28 days at a saturation level of about 5 μg/L to fathead minnows, *Lumbriculus*, *Hyalella*, and *Gammarus*.

## Methods and Materials

### *Sediment*

The sediment, 150 L, was collected at a bottom depth of 3.5 m from Porter Lake, a small oxbow about 13 km south of Corvallis, OR, with Ekman and PONAR grab samplers. Overlying water in the samplers was decanted during collection. The sediment was placed in large polyethylene bags in ice chests and kept refrigerated at 5°C until needed. It was mixed in bulk before being dispensed to individual spiking flasks. Particle size distribution indicated that over 70% passed through a 63 μm mesh sieve (Malueg *et al.* 1986). The sediment contained 4% organic carbon, 30% dry solids, and had a specific gravity of about 1.4.

The sediment was spiked by shaking it in flasks partially coated with HCB. The HCB was dissolved in pentane and coated on the inside of 2-L Erlenmeyer flasks up to a height of 3 cm using a rotary evaporator at 40°C. Wet sediment (1,350 to 1,750 g) was added to the flasks which were shaken at 20°C at 100 oscillations per min until used or stored. The HCB level, which reached equilibrium in 6 days, did not change even after 77 days of shaking followed by up to 244 days of cold storage at 5°C in the spiking flasks (Table 1).

### *Water*

Test water was obtained from a laboratory well near the Willamette River at Corvallis, OR. The water is characteristically soft with low levels of dissolved constituents. It was amended to a total hardness of 200 mg/L as $CaCO_3$ by adding $CaSO_4 \cdot 2H_2O$, $MgCl_2 \cdot 6H_2O$, $NaHCO_3$, and $KHCO_3$ in a modification of the method described by the American Public Health Association (1985) to eliminate a potential depression of pH evident in preliminary tests with unbuffered well water.

**Table 1.** Hexachlorobenzene-spiked sediment used in tests

| Test | Nominal HCB spike μg/g dry solids[a] | Days on shaker[b] | Days cold storage before use | Measured HCB, μg/g dry solids $\bar{x} \pm SE$ | |
|---|---|---|---|---|---|
| | | | | Test start | Test finish |
| *Lumbriculus* | 56.7 | 77 | 107 | 77 ± 1 | 162 ± 2 |
| *Hyalella* | 49.7 | 77 | 190 | 76 ± 1 | 107 ± 11 |
| *Gammarus* | [c] | 77 | 244 | 56[c] | 71 ± 6 |
| Fathead 1 | 51.7 | 12 | 0 | 58 ± 3 | 85 ± 8 |
| Fathead 2 | 51.7 | 77 | 41 | 69 ± 1 | 112 ± 9 |

[a] Estimated amount of HCB coated on shaker flasks per gram of sediment dry solids

[b] Equilibrium reached in 6 days

[c] Mixture of approximately 17% 70 μg HCB/g dry solids and 83% 50 μg HCB/g dry solids. Replicate data not available at start of test

## *Test Organisms*

All test organisms were cultured in flowing well water at 20°C, kept on a 16 hr light:8 hr dark cycle, and fed HCB-free food. Stock cultures of fathead minnows, *Pimephales promelas* Rafinesque, were fed brine shrimp and Oregon Moist pellet fish food. Stock cultures of the amphipods *Hyalella azteca* (Saussure) and *Gammarus lacustris* Sars were maintained in aquaria with dried maple leaves and pelleted rabbit food. The oligochaete worm, *Lumbriculus variegatus* (Müller), was reared and collected from the detritus in the bottom of selected *Hyalella* aquaria. For the sake of brevity and common usage, the test species will be referred to in this paper as fathead minnows, *Hyalella*, *Gammarus*, and *Lumbriculus*. Fathead minnows and *Gammarus* were transferred into holding containers a few hours before they were added to the test aquaria. *Lumbriculus* and *Hyalella* were held several days before they were added because of the length of time needed to collect sufficient organisms.

Feeding rates and biomass loading are described in Table 2. Waste food and detritus, where visible, were removed daily from the bottom of the aquaria with minimal disturbance to the sediment.

## *Test Conditions*

Organisms were exposed to HCB in the water of 20 L aquaria with and without a layer of HCB-spiked sediment (Figure 1). Maintenance of similar and constant concentrations of HCB in the water was accomplished by recirculating the water through HCB-packed columns. The amount of HCB bioconcentrated by the organisms was measured periodically throughout the accumulation and depuration phases of the tests. Tests were run in triplicate except as noted.

Hexachlorobenzene was coated on the 5 mm dia. glass beads used in the recirculation columns by rotating 200 g of beads in 250 ml pentane containing 0.7 g HCB in a 2000 ml Erlenmeyer flask on a rotary evaporator. The flask was adjusted to an angle sufficient to permit the beads to roll while evaporating the pentane. Rolling continued for 5 min after visible evidence of liquid evaporation had occurred.

Testing was conducted in an environmental room in ventilated plexiglass cabinets under the same light and temperature conditions as the stock cultures. Methods outlined by the American Society for Testing and Materials (1984a) were generally used as guidelines for the bioconcentration tests with modifications made for recirculating instead of flow-through tests.

The water in each aquarium was recirculated with a bubble lift-pump (Figure 1) through a 33 cm × 2.1 cm ID glass column containing 100 ml of HCB-coated 5 mm glass beads. Oil-free diaphragm-type aquarium pumps were used for an air source. The air for the lift pump first passed through an activated carbon column to remove impurities and then through an HCB-air column to saturate the air with HCB. The HCB thus gained by the air lessened the potential of the lift pump to strip HCB from the water. The air-flow rate to each aquarium was adjusted with a separate metal plug valve. The recirculated water flow rate was 100 ml/min for the fathead minnow tests and 175 ml/min for the others. All glass tubing connections were made with teflon sleeves. Eight 10-cm petri dishes, covering about 80% of the surface area, were floated on the water surface of each aquarium to retard evaporation and HCB loss. In addition, the top of each aquarium was covered with aluminum foil. A stainless steel screen with 0.75 mm openings was fitted around the lift pump in the *Hyalella* experiment to prevent the organisms from gaining access to the HCB-column and clogging the tubing.

Sediment was added to half of the aquaria in every experiment, except for *Lumbriculus* where the sediment was contained in glass dishes on the bottom of the aquaria. The sediment was allowed to settle overnight and water was carefully added with the use of a baffle to minimize disturbance. The sediment aquaria in the fathead minnow tests received 1.2 L of sediment (1.5 cm deep) and 16.8 L of water. The *Hyalella* and *Gammarus* sediment aquaria received 0.8 L of sediment (1.0 cm deep) and 17.2 L of water. The *Lumbriculus* dishes were made of cut 250-ml beakers 4.3 cm high and contained 50 ml of sediment. Thirteen dishes of sediment were placed in each aquarium after the water was added. Total volume of sediment in the 13 dishes was 0.65 L; the volume of added water was 17.2 L. White quartz sand (20 mesh) was used as a control substrate for *Lumbriculus* in dishes without sediment. All aquaria without sediment received 18 L of water.

Recirculation of the water through the HCB columns was started in the fathead minnow and *Lumbriculus* tests after two days of sediment settling, and after 1 and 3 days for the *Gammarus* and *Hyalella* tests, respectively. The concentration of HCB in the water reached a steady state within 7 days. Organisms were added within the next 11 days.

The tests were run for a 28-day accumulation period (except for 30-day for *Gammarus*) followed by a 15- to 28-day depuration period in clean water. The *Hyalella* test had no depuration phase. Control vessels with and without sediment, but without HCB, were run in each experiment.

All organisms removed for tissue analysis were held for one day in beakers of HCB-free test water to allow the gut to clear. The depuration phase was conducted by placing remaining animals in aerated sediment-free 4-L jars, changing the water three times a week.

Water samples (100 ml) to characterize temporal changes in HCB water concentration were collected 6 to 8 times during accumulation

**Table 2.** Feeding rates and animal biomass loading in experimental aquaria[a]

| Test | Feeding rate | | Initial biomass/ aquaria, g | Initial biomass loading, g/L[b] | |
|---|---|---|---|---|---|
| | % Body weight/day | g/day/ Aquarium[b] | | Aquaria without sediment | Aquaria with sediment |
| *Lumbriculus* | 2.0 | 0.05 | 2.6 | 0.14 | 0.15 |
| *Hyalella* | 5.0 | 0.08 | 1.7 | 0.09 | 0.10 |
| *Gammarus* | 5.0 | 0.25 | 5.0 | 0.28 | 0.29 |
| Fathead 1 | 3.0 | 0.40 | 13.5 | 0.80 | 0.75 |
| Fathead 2 | 3.0 | 0.08 | 2.6 | 0.14 | 0.15 |

[a] *Lumbriculus* and fatheads were fed Oregon Moist Pellet fish food; *Hyalella* and *Gammarus* were fed pelleted rabbit food
[b] Organisms were removed periodically from aquaria for tissue analysis, so amount fed and loading decreased over time

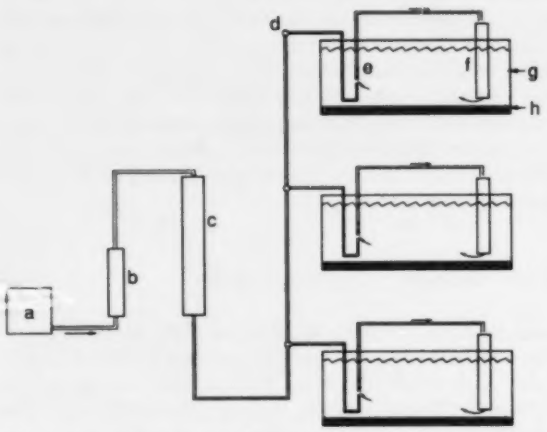


**Fig. 1.** Test apparatus. a. air pump; b. activated carbon column; c. HCB air column; d. valve; e. bubble lift-pump; f. HCB water column; g. aquarium; h. sediment

phases and 4 to 5 times during depuration phases. Tissue samples, at least 0.3 g, were collected at the same time as the water samples. Water volume loss due to evaporation (5%) was replaced by reverse osmosis water. Hardness-adjusted well water was added to compensate for loss due to sampling for HCB and other constituents. Samples (50 to 100 ml) for hardness, alkalinity, and conductivity were collected at the beginning, midpoint, and end of the accumulation and depuration phases. Samples for nitrate nitrogen, ammonia, and organic carbon were collected five times during these phases.

## *Analytical Procedures*

One-hundred milliliter samples of water were extracted for HCB with ethyl acetate via liquid–liquid extraction with a magnetic stirrer (Ripley *et al.* 1974; Muir and Baker 1976). The extracts were concentrated, using a Kuderna-Danish concentrator (Federal Register 1973). Samples were analyzed, using fused silica capillary column gas chromatography with electron capture detection on a Tracor 570 or Hewlett-packard 5730 GC operated in the split mode at a split ratio of 50–75:1. Sediment samples were analyzed for HCB by diluting 5 g wet sediment with 100 ml reverse osmosis water and extracting the sediment-water mixture with ethyl acetate and analyzing as above. Animal tissue was frozen at −6°C after collection. Tissue subsamples of about 300 mg were extracted in a Potter-Elvehjem tissue grinder (USEPA 1980) with ethyl acetate. The extracts were brought to volume and analyzed by gas chromatography as above. Standards were made from 99% HCB obtained from Supelco, Inc.[1] Five or six standards were run at the beginning of an analytical run with a single standard analyzed after approximately every six samples.

Detection limits for HCB in water and sediment were 0.06 μg/L and 0.1 μg/g, respectively. The detection limit for HCB in tissue ranged from 0.1 to 2.0 μg/g for *Lumbriculus*, 0.3 to 0.7 μg/g for *Gammarus*, 1.0 to 3.0 μg/g for the fathead minnows, and 2.0 μg/g for *Hyalella*. The specific recovery of HCB in spiked water samples from test vessels with and without sediment, based on extraction and injection, was 96.4 ± 1.1% ($\bar{x}$ ± SE, N = 53). The mean coefficient of variation for pairs of water replicates was 10.5 (N = 56 pairs). Specific recovery for spiked tissue from exposed organisms was 104.9 ± 2.1% ($\bar{x}$ ± SE, N = 15), with a mean coefficient of variation for pairs of replicates of 21.6 (N = 46 pairs). The mean coefficient of variation for pairs of sediment replicates was 9.1 (N = 10 pairs).

Dissolved oxygen and pH were measured by electrode, two to three times per week. Total hardness was calculated from calcium and magnesium content as measured with an inductively coupled plasma-atomic emission spectrometer (McQuaker *et al.* 1979). Turbidity was measured with a Hach 18900 ratio turbidimeter. Total carbon in the sediment was determined by combustion and measurement of carbon dioxide (Paterson 1973). Total sediment inorganic carbon was determined by a modification of the calcimeter method of Allison and Moodie (1965). Total organic carbon was by difference. Total organic carbon in the water was determined by coulometric detection (ASTM 1982). Total un-ionized ammonia was estimated by graphical interpolation using tables of percent un-ionized ammonia in aqueous ammonia solutions (USEPA 1976). Alkalinity samples were preserved with mercuric chloride and analyzed for total inorganic carbon (bicarbonate ion) (ASTM 1984b). The bicarbonate was converted to equivalent alkalinity. All other chemical analyses were conducted according to USEPA methods (USEPA 1979).

Animal wet weight was determined by low temperature immobilization of the organisms followed by blotting for 1 min on filter paper before weighing. Dry weight was determined by drying the organisms for 6 hr at 105°C, cooling in a desiccator, and weighing (USEPA 1973).

Soluble constituents were operationally defined as those present in the supernatant after centrifugation at 20,000 rpm (48,200 × G) for 30 min in 50-ml stainless steel tubes.

[1] Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

### *Calculations*

Concentrations of HCB in water were maintained relatively constant by passing the water through a column of HCB-coated glass beads. However, some fluctuations occurred. Therefore, a time-averaged concentration was used as the measure of exposure concentration. Values for the accumulation and depuration phases were determined separately according to the following:

$$\overline{C}j = \frac{\sum_{i=1}^{j} \left(\frac{C_i + C_{i-1}}{2}\right) \cdot (t_i - t_{i-1})}{t_j} \quad (1)$$

where $\overline{C}_j$ = Exposure concentration at the time $t_j$.
$C_i$ = Concentration at the time $t_i$.
$t_i$ = Time (days) of measurement.

The observed bioconcentration factor was determined by:

$$BCF = CB/CW \quad (2)$$

where $C_B$ = Concentration in biota.
CW = Time-averaged concentration in water, $\overline{C}_j$.

The observed accumulation relative to sediment (Pavlou and Weston 1983) was determined by:

$$ARS = CB/CS \quad (3)$$

where $C_B$ = Concentration in biota.
$C_S$ = Concentration in sediment.

Significant differences between means or testing if one mean was significantly greater than another was done with approximate two- or one-tailed *t* tests which did not assume equal variances between populations (Ryan *et al.* 1985).

## Results

### *Water Quality and Mortality*

Water quality parameters followed a similar pattern in the five experiments. Dissolved oxygen was high, generally averaging between 8 and 9 mg/L, and only dropped as low as 6 mg/L in the sediment aquaria of the first fathead aquarium experiment. The pH ranged from 7.1 to 8.9 and was usually similar between vessels with and without sediment.

Turbidity increased substantially in most tests after organisms were added to aquaria containing sediment. Turbidity in aquaria without sediment ranged from 0.3 to 1.0 nephelometric turbidity units (NTU). Turbidity in aquaria with sediment ranged from 1.8 NTU (*Lumbriculus*) to 226 NTU (fathead minnow test 1). Hardness, alkalinity, and conductivity were all higher in aquaria without sediment. The mean values for these parameters (ranging from a low in aquaria with sediment to a high in aquaria without sediment) were, respectively, 140 to 202 mg/L, 10 to 120 mg/L, and 558 to 682 microsiemens (μS). Conversely, nitrate and total organic carbon were higher in aquaria with sediment and increased over time. Mean nitrate ranged from a low of 0.4 mg/L in aquaria without sediment to a high of 3.9 mg/L in aquaria with sediment. Mean total organic carbon ranged from a low of 0.4 mg/L in aquaria without sediment to a high of 11.2 mg/L in aquaria with sediment. Total organic carbon in the *Hyalella* aquaria with animals present ranged from 4.2 to 5.0 mg/L; the *Hyalella*-free aquaria contained only 1.4 to 2.2 mg/L. Mean un-ionized ammonia was low and ranged from <1 to 8 μg/L in all aquaria, which is below the 4-day average water quality criteria concentration of 35 μg/L for salmonids or other sensitive coldwater species for the maximum pH observed in these experiments (USEPA 1985).

The HCB-coated glass beads in the *Hyalella* experiment were analyzed at the conclusion of the test. At the start, each bead in the water column had been coated with approximately 490 μg of HCB. The coating was reduced to about 360 μg per bead in both sediment and water vessels at the conclusion. There was no loss of HCB from beads in the *Hyalella*-free aquaria in this experiment.

Mortality in the *Lumbriculus* experiment was undetectable as the numbers of organisms had increased by the end of the test (Table 3). Fathead mortality was low, ranging from 1 to 11%. *Hyalella* and *Gammarus* (old adults) mortality was much higher, ranging from 37 to 50%. There were many newborn *Hyalella* present at the end of the tests, however. *Lumbriculus* lost weight during the depuration phase, while *Gammarus* and the fathead minnows increased in biomass over time (Table 3).

### *Water, Sediment, and Tissue HCB*

Concentrations of soluble HCB in the water of the different tests were often near the reported saturation level of 5 μg/L (Chiou *et al.* 1982) (Figure 2). Soluble HCB concentrations responded differently in the various experiments after the introduction of organisms (Figure 2). Concentrations remained essentially unchanged in the *Lumbriculus* test. HCB increased in the aquaria without sediment in the *Hyalella* test. In the *Gammarus* test, the HCB initially decreased with time then increased. There was an initial decrease in HCB with a subsequent leveling in the first fathead minnow test. In the second fathead minnow test, HCB in the aquaria with sediment decreased and then leveled off, while in the aquaria without sediment there was an initial low peak before leveling off. There were no significant differences between soluble HCB levels in the aquaria with and without sediment at 28 days except in the *Gammarus* test (Table 4, $p = <0.10$).

A third set of replicates with and without sediment in the *Hyalella* aquarium experiment was run without organisms, allowing the effect of the organisms on HCB content to be analyzed. There were no differences in soluble HCB in aquaria with or without *Hyalella*.

The sediments exhibited substantial increases in HCB ranging from a 40% increase in the *Hyalella* experiment to a 100% increase in the *Lumbriculus* experiment (Table 1). There was no gain of HCB in the sediment of the *Hyalella* free aquarium.

Tissue HCB uptake over time was very similar between the aquaria with and without sediment in the *Gammarus* and first fathead tests (Figure 2). Tissue HCB content in the second fathead minnow test uptake phase was always higher in the aquaria without sediment. *Lumbriculus* tissue HCB levels were higher in the aquaria without sediment after 4 days. Tissue HCB levels were higher in the aquaria without

**Table 3.** Organism mortality and weight

| Test | No. organisms added per vessel | % Mortality per vessel[a] $\bar{x} \pm SE$ | Mean wet weight per individual, mg[b] Start | End accumulation phase | End depuration phase |
|---|---|---|---|---|---|
| *Lumbriculus* | 520 | (816 ± 22)[c] | 5 | 4.9 | 3.6 |
| *Hyalella* | 560 | 49.6 ± 1.8[d] | 3 | 4.3 | — |
| *Gammarus* | 45 | 37.3 ± 2.5 | 110 | 130 | 120 |
| Fathead 1 | 50[e] | 11.4 ± 4.6 | 270 | 330 | 500 |
| Fathead 2 | 55 | 1.0 ± 0.3 | 50 | 70 | 130 |

[a] Test and control mortality combined; aquaria data adjusted for tissue removal
[b] Determined by total weight/number of individuals
[c] Number of survivors plus total used for tissue analysis
[d] Many newborn in vessels
[e] Controls had 24 per vessel

sediment than in aquaria with sediment for *Hyalella*, except for the final day when they were the same. Tissue HCB steady-state conditions in aquaria with sediment were apparently reached in the *Lumbriculus* test and were approached in the *Gammarus* and fathead minnow tests. Tissue steady-state conditions were not reached for these organisms in aquaria without sediment.

Tissue HCB concentrations at 28 days (Table 4) were significantly higher in the *Lumbriculus* aquaria without sediment ($p = <0.05$) and in the *Gammarus* and second fathead minnow tests ($p = <0.10$). There was no significant difference in tissue HCB levels ($p = >0.05$) between aquaria with and without sediment in the *Hyalella* and the first fathead minnow tests.

### *Bioconcentration*

Bioconcentration factors (BCF) based on total and soluble HCB are displayed in Table 4. There were no cases where BCFs in aquaria with sediment were significantly higher than in aquaria without sediment. The BCFs were significantly higher in the aquaria without sediment in the *Lumbriculus* ($p = <0.05$) and second fathead minnow tests ($p = <0.10$).

The accumulation of HCB in the organisms relative to the amount in the sediment (ARS) is shown in Table 5. The ARS values generally paralleled trends in the bioconcentration factors and tissue HCB levels. *Lumbriculus*, for instance, with an ARS of only 2.1, had the lowest tissue HCB level and bioconcentration factor (Table 4).

## Discussion

Bioconcentration factors were calculated from tissue data rather than derived from a kinetic model (Branson *et al.* 1975) because of a desire not to extrapolate beyond observed data and the possibility that the addition of sediment might complicate the system such that a two compartment model as commonly used might be invalid. Rather than develop estimates of HCB accumulation, the major goal of this study was to compare measured HCB uptake in two systems having similar HCB water concentrations but with one also containing spiked sediment.

Water recirculated through a column containing a test chemical has been used to expose fish in bioaccumulation experiments (Bruggeman *et al.* 1984). Giam *et al.* (1980) recirculated aquarium water through a column containing layers of glass wool and HCB-spiked sand. Our columns were more open and used glass beads supported on 30-mesh stainless steel to prevent clogging from fines released from the aquaria with sediment. However, this may have permitted the release of particulate HCB and increased the potential for recirculating sediment and other particles to erode and adsorb HCB from the columns.

The loss of HCB from the beads, as measured in the *Hyalella* experiment, and the increase of sediment HCB in all of the experiments, suggests that the organisms were subjected to several sources of HCB: (1) water; (2) sediment bed; (3) eroded HCB particulates; and (4) HCB adsorbed onto recirculating sediment, food, or fecal particles. The HCB erosion in both the aquaria with and without sediment was about the same, so the organisms in both systems were probably subjected to equal amounts of eroded HCB. The lack of increase in sediment HCB in the *Hyalella*-free aquarium was likely associated with lower levels of TOC as compared to aquaria with animals. Water with lower levels of TOC would have less of an affinity for HCB as it passed through the HCB column, resulting in less HCB adsorbed by the sediment. The increased sorption of organic compounds to suspended solids of increased levels of organic carbon has been evaluated by Staples *et al.* (1985).

Ingestion of sediment-bound contaminants can contribute to an increased body burden in some cases. The deposit feeding marine bivalve, *Abra nitida*, accumulated more HCB in the presence of added turbidity which was attributed to the ingestion of HCB-bound particles (Ekelund *et al.* 1987). *Hyalella* was reported to accumulate more anthracene in the presence of sediment (Landrum and Scavia 1983). *Lumbriculus* is a deposit feeder and might be expected to exhibit a greater accumulation of HCB when in the presence of spiked sediment. Fathead minnows feed upon detritus, insects, and algae. Forbes and Richardson, cited in

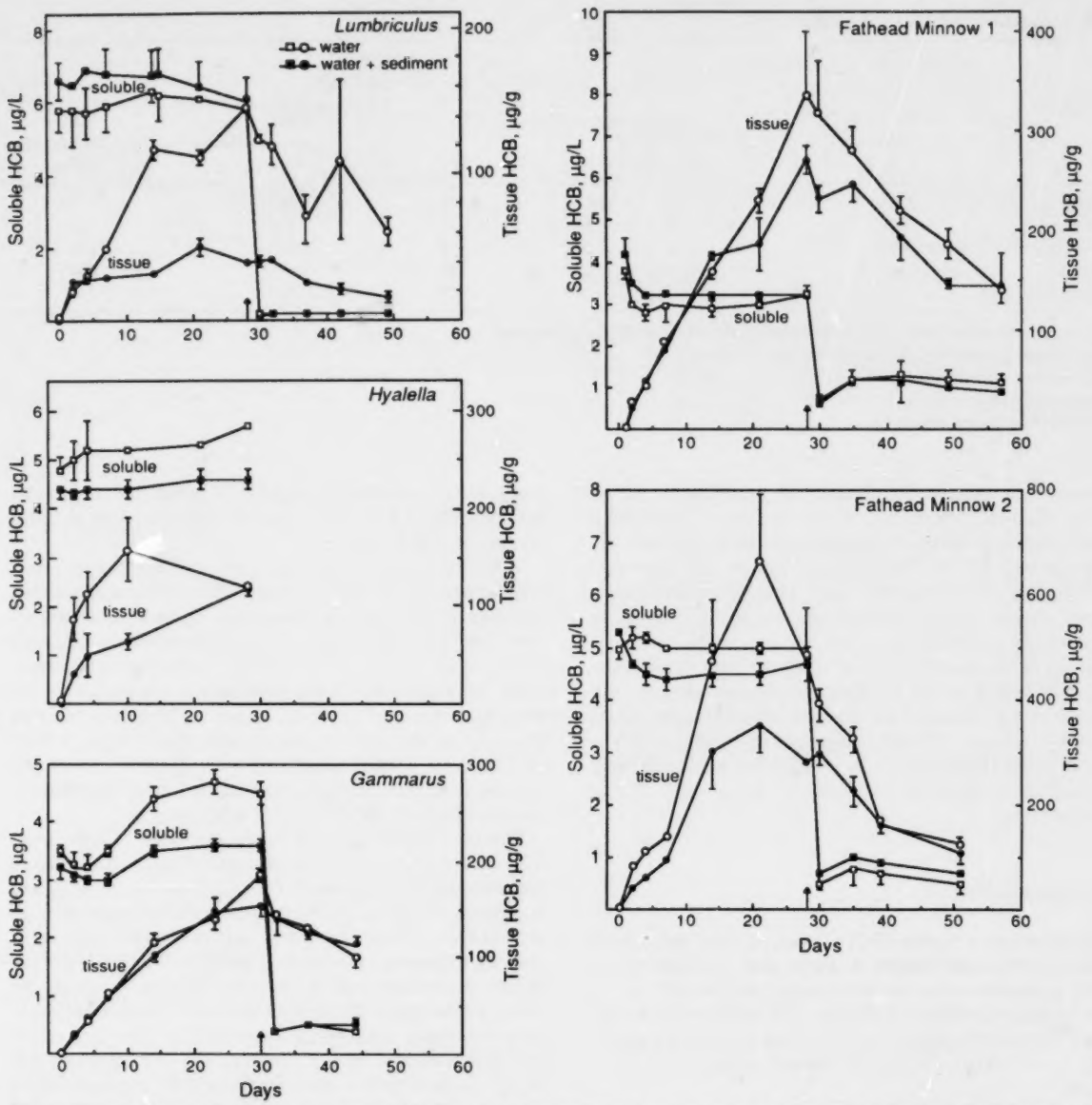


**Fig. 2.** Water column HCB (µg/L) and tissue HCB (µg/g) in water-only and sediment-water tests. The arrow indicates the end of the accumulation and beginning of the depuration phase

Smith (1979), reported mud in the gut, which indicates the potential for exposure to ingested sediments. Contributions to body content by ingestion, however, were not apparent in our experiments. If the relative contributions of possible eroded HCB particulates and associated recirculating food and fecal pellets are assumed to be the same whether or not sediment was present, then the lower body burden of HCB in organisms in aquaria with sediment suggest that the HCB was going into the sediment rather than the organisms. This is supported by an increase of sediment HCB over time. It appears that the sediment had a greater affinity for HCB than did the organisms. This may be important in evaluating organism response to both a contaminated sediment bed in addition to other sources.

Accumulation relative to sediment (ARS) was suggested by Pavlou and Weston (1983) as a quasi-equilibrium constant that provides a tool for estimating the potential accumulation in the biota from the sediment. They emphasized that ARS, while not revealing bioaccumulation mechanisms, provides a rough indication of accumulation patterns specific to classes of compounds. Oliver (1984) reported 110 day HCB concentration factors (dry weight concentration in worms/dry weight concentration in sediment) for oligochaetes ranging from 0.26 to 0.54 in "naturally" contami-

**Table 4.** Hexachlorobenzene in water and tissue at end of 28-day accumulation phase (N = 3, $\bar{x}$ ± SE)

| Test | | Water HCB, µg/L[a] Total | Soluble | Tissue HCB µg/g | BCF[b] |
|---|---|---|---|---|---|
| *Lumbriculus* | W[c] | 20.5 ± 2.7[e] | 5.8 ± 0.1 | 149 ± 3[f] | 25,100 ± 800[f] |
| | S[d] | 13.6 ± 1.1 | 6.0 ± 0.6 | 39 ± 2 | 6,700 ± 2,900 |
| *Hyalella*[g] | W[h] | 37.8 | 5.7 | 118 ± 0.5 | 20,700 |
| | S | 15.1 ± 4.6 | 4.6 ± 0.2 | 118 ± 6 | 26,000 ± 2,600 |
| *Gammarus*[i] | W | 8.5 ± 0.8 | 4.5 ± 0.2[e] | 185 ± 7[e] | 41,200 ± 3,000 |
| | S | 7.9 ± 0.4 | 3.6 ± 0.1 | 153 ± 13 | 42,400 ± 2,900 |
| Fathead 1 | W | 11.0 ± 5.0 | 3.5 ± 0.2 | 337 ± 63 | 95,400 ± 9,800 |
| | S | 11.4 ± 1.2 | 3.2 ± 0.1 | 272 ± 14 | 83,800 ± 2,900 |
| 2 | W | 8.6 ± 0.7 | 5.0 ± 0.1 | 468 ± 84[e] | 93,800 ± 16,900[e] |
| | S | 8.9 ± 0.2 | 4.7 ± 0.3 | 282 ± 2 | 49,200 ± 6,900 |

[a] Time-averaged water concentration
[b] Each bioconcentration (BCF) value is the mean of individual wet weight biota HCB concentrations/individual water HCB concentrations for each set of water and tissue replicates. Based on soluble (centrifuged samples) HCB
[c] W = vessels without sediment
[d] S = vessels with sediment
[e] Significant difference between W and S ($p = <0.10$)
[f] Significant difference between W and S ($p = <0.05$)
[g] N = 2
[h] Replicates in aquaria without sediment not included due to possible chemical interference
[i] 30 day accumulation phase

**Table 5.** Comparison of tissue hexachlorobenzene accumulation relative to sediment concentration after 28 days (N = 3, $\bar{x}$ ± SE)

| Test | No. aquaria | HCB, µg/g Tissue[a] | Sediment[b] | ARS[c] |
|---|---|---|---|---|
| *Lumbriculus* | 3 | 258 ± 16 | 120 ± 42 | 2.1 ± 0.1 |
| *Hyalella* | 2 | 560 ± 26 | 92 ± 16 | 6.1 ± 0.3 |
| *Gammarus*[d] | 3 | 635 ± 53 | 64 ± 8 | 9.9 ± 0.8 |
| Fathead 1 | 2 | 1,056 ± 52 | 72 ± 12 | 14.6 ± 0.8 |
| Fathead 2 | 3 | 1,093 ± 8 | 90 ± 22 | 12.1 ± 0.1 |

[a] Based on dry weight as a percentage of wet weight (*Lumbriculus* 15%, *Hyalella* 21%, *Gammarus* 24.1%, fatheads, 25.8%)
[b] Mean of initial and final analyses, based on dry solids
[c] ARS = accumulation relative to sediment (concentration biota/concentration sediment)
[d] 30 day accumulation period

nated sediments. Those are considerably less than the ARS value of 2.1 (Table 5) from our spiked sediments. Oliver's sediments contained from 0.051 to 0.360 µg/g HCB while ours averaged from 72 to 120 µg/g. The ARS results in these test are only suggestive because the sediment concentrations were not at steady state; the general trends, however, are significant. *Lumbriculus*, an organism that lives in intimate contact with the sediment, accumulated far less tissue HCB relative to sediment HCB concentrations than did the fathead minnows which are commonly associated with the water column. This may support the importance of the water as a transfer route.

Mortality in the *Hyalella* test (Table 3), while high, was probably a result of using mature reproducing adults at the end of their life cycle. Numerous newborn organisms in the test aquaria at the end of the experiments also suggested this mode of mortality rather than some toxic component. *Gammarus* mortality was also likely due to using old animals. Increases in numbers through asexual reproduction in the *Lumbriculus* tests masked any mortality that might have occurred. Increased mortality for the larger fish in the first fathead minnow test could be age-related.

## Conclusions

The water recirculating system in these tests had the advantage of rapid HCB delivery, high capacity and ease of sampling, but the disadvantage of the possiblity of particulate HCB being dislodged or easily eroded from the column and transported directly to the biota or sediment.

None of the tests indicated sediment was a more important HCB exposure route than the water. The organisms in the aquaria with sediment were exposed to a higher propor-

tion of adsorbed HCB because of the sediment bed and recirculating sediment particles. Even with this added source of HCB, these organisms accumulated less HCB than the organisms in sediment-free aquaria, suggesting that the sediment was a more efficient sink for HCB than the organisms. This is supported by the gain of sediment HCB over time. These conditions are especially evident in the case of *Lumbriculus*. Total organic carbon levels in the water, recirculation of suspended HCB particulates, and HCB-bound food and fecal particles were all important factors in evaluating HCB uptake in these laboratory systems. These factors should also be considered in the design of any water/sediment test system evaluating the relative uptake of organic xenobiotics.

Field conditions where organic xenobiotics are bound to sediments are complex and not easily measured. It may not be possible to protect organisms in the field using laboratory data, but the data can be very useful when interpreting field-collected information. The results of these laboratory experiments may suggest possible organism response in a field situation where they might be exposed to a contaminated sediment bed in addition to a source of contaminated particulates. Experiments of this type may also aid in the evaluation of certain components of waste discharges by revealing toxicity and bioconcentration potential and need for remedial action.

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